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Effects of Anesthetics on the Structure of a Phospholipid Bilayer: Molecular Dynamics Investigation of Halothane in the Hydrated Liquid Crystal Phase of Dipalmitoylphosphatidylcholine

Kechuan Tu,*# Mounir Tarek,*§ Michael L. Klein,* and Daphna Scharf*¶

*Center for Molecular Modeling, Department of Chemistry, University of Pennsylvania Philadelphia, Pennsylvania 19104-6323; #Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-0446; §NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, Maryland 20899; and ¶Department of Anesthesia, Medical Center, University of Pennsylvania, Philadelphia, Pennsylvania 19104-4283 USA

ABSTRACT We report the results of constant temperature and pressure molecular dynamics calculations carried out on the liquid crystal (L_α) phase of dipalmitoylphosphatidylcholine with a mole fraction of 6.5% halothane (2-3 MAC). The present results are compared with previous simulations for pure dipalmitoylphosphatidylcholine under the same conditions (Tu et al., 1995. *Biophys. J.* 69:2558–2562) and with various experimental data. We have found subtle structural changes in the lipid bilayer in the presence of the anesthetic compared with the pure lipid bilayer: a small lateral expansion is accompanied by a modest contraction in the bilayer thickness. However, the overall increase in the system volume is found to be comparable to the molecular volume of the added anesthetic molecules. No significant change in the hydrocarbon chain conformations is apparent. The observed structural changes are in fair agreement with NMR data corresponding to low anesthetic concentrations. We have found that halothane exhibits no specific binding to the lipid headgroup or to the acyl chains. No evidence is obtained for preferential orientation of halothane molecules with respect to the lipid/water interface. The overall dynamics of the lipid-bound halothane molecules appears to be reminiscent of that of other small solutes (Bassolino-Klimas et al., 1995. *J. Am. Chem. Soc.* 117:4118–4129).

INTRODUCTION

Investigation of membrane lipids and their interaction with general anesthetics (GAs) has been the subject of extensive study in the quest for the molecular mechanism of anesthesia. It was in 1901 that Overton and Meyer (Overton, 1901; Meyer, 1901) presented their famous correlation between the potency of inhaled GAs and their corresponding solubility in olive oil. They concluded that GAs dissolve in (brain) cell lipids and thereby cause anesthesia due to decreased cell functions. Anesthetic lipid solubility became the basis for the lipid theory of narcosis (Meyer, 1937; Miller and Smith, 1973; Curatola et al., 1991). This theory has been supported by observations of alteration in membrane structure, and/or phase, at sufficiently high anesthetic concentrations (Miller, 1985; Trudell et al., 1973; Trudell, 1977; Pringle and Miller, 1979; Suezaki et al., 1985; Cullis et al., 1980; Koehler et al., 1980). More recently NMR and NOE studies focused on the possible inhomogeneous distribution of anesthetics and nonimmobilizers in model lipid bilayers (Tang et al., 1997; Xu and Tang, 1997; North and Cafiso, 1997; Baber et al., 1995).

The role of the membrane proteins in mediating anesthesia has attracted much attention since the early studies by Franks and Lieb (1984), which pointed at direct binding of anesthetics to a protein target. Indeed, a number of experimental studies have demonstrated saturable binding of GAs to water-soluble peptides and proteins (Eckenhoff and Johansson, 1997, and references therein; Mihic et al., 1997, and references therein; Franks and Leib, 1990, 1997, and references therein; Harris et al., 1995; Johansson et al., 1995). These studies suggest that the association of GAs with certain proteins, possibly channels, can alter their activity. However, the nature of the molecular interactions that underly the function modifications remain obscure. Moreover, even the structural and property modifications of the membrane lipid bilayer in the presence of clinically relevant concentrations of GAs are still unclear. Early work based on x-ray and neutron diffraction studies of anesthetics in dimyristoyl lecithin/cholesterol bilayers concluded that there is no significant change in the bilayer structure (Franks and Lieb, 1979). Simon et al. (1979) and Kaneshina et al. (1981), based on the insensitivity of the partition coefficients to the length of the acyl chain, concluded that anesthetics may not penetrate into the lipid core region. Lieb et al. (1982), using Raman spectroscopy, showed that at clinical concentrations halothane has no effect on the hydrocarbon chain conformation of a dimyristoylphosphatidylcholine (DMPC)/cholesterol multilamellar suspension. Craig et al. (1987) used the same technique to study halothane in dipalmitoylphosphatidylcholine (DPPC) and reported similar findings. They concluded that the interaction between halothane and the lipid bilayer occurs in the headgroup region. This idea of the water interface being the primary localization of the anesthetic was also supported by Shieh et al., (1976) in a proton NMR study of 1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine (DHDPC) liposomes

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Address reprint requests to Dr. Michael L. Klein, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323. Tel.: 215-898-8571; Fax: 215-898-8296; E-mail: klein@lrsm.upenn.edu.

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and Tsai et al. (1987) in an IR study of halothane in reverse DMPC/water/benzene micelles. The same IR technique was also used for halothane in DPPC vesicle membranes (Tsai et al., 1990). At high concentration, the anesthetic was found to induce significant changes in the stretching frequencies associated with some of the headgroup moieties, which suggested that the primary localization was the lipid headgroup (Tsai et al., 1990; Ueda et al., 1986). NMR data were interpreted to show preferential orientation of some anesthetics with respect to the lipid/water interface of surfactant micelles, which are characterized by charged headgroups (Yoshida et al., 1989).

Different conclusions have been drawn from NMR studies of anesthetics in a variety of lipid media. Trudell et al. (1976) used ^{19}F-NMR for halothane in egg PC to study the molecule's accessibility to the aqueous phase of the bilayer. They found that the anesthetic rapidly exchanges between the aqueous and the bilayer phase and has equal access to the bilayer interface and the hydrocarbon region. Baber et al., (1995) used deuterium NMR and NOE measurements to study the structure of a palmitoyloleoylphosphatidylcholine multilammelar dispersion containing halothane. Their results indicate that the anesthetic resides in the hydrocarbon region of the membrane, with a slight preference for the membrane solution interface, but is not found in high concentration within the charged headgroup region. In a subsequent study (North and Cafiso, 1997) the same group used ^{19}F-NMR techniques to probe the location of the anesthetics. The fluorine chemical shift obtained for halothane in DMPC vesicle suspensions was found to be more similar to that of the molecule solvated in water than in a liquid hydrocarbon. Because the halothane experiences an environment that is on average similar to that of water, the natural conclusion was that the halothane resides preferentially in the interfacial domain. Similar observations have been made in studying ^{19}F-NMR spectra of halogenated compounds in phosphatidylcholine suspensions (Tang et al., 1997). They concluded that anesthetics distribute preferentially to regions of the membrane that permit easy contact with water.

The study of general anesthetic distribution in membrane can be viewed in more general terms of the permeability of the water/lipid interface to small solutes and the dynamics of small solute diffusion in inhomogeneous and anisotropic media. Notable theoretical studies characterized the transport in simple liquids as movement of molecules to voids (Cohen and Turnbull, 1959; Frankel, 1946). Small diffusing molecules were characterized as spending most of the time confined to a "cage" bound by the immediate neighboring molecules. The displacement of a molecule contained in such a "cage" was enabled by hopping between empty voids opening in the liquid, which were due to density fluctuations. Considering membranes, Lieb and Stein (1969, 1971) suggested that the lipid region should be regarded as a "soft polymer." Small molecule diffusion across membranes should be facilitated by dynamics of free volume pockets in the membrane, into which the solute can "jump" (Franks and Lieb, 1981).

Computer simulations can test the theoretical notions and complement experimental techniques to further the conceptual understanding of the solute dynamics in lipid bilayers. The equilibrium transport properties and distribution are of seminal importance in drug delivery. The distribution and solute dynamics are a key to locating probable sites for drug interactions. Pohorille and Wilson (1996) concentrated on the free energy profiles for the transfer of small molecules and some anesthetics. They demonstrate the inhomogeneous distribution of these solutes at interfaces of water/hexane (Chipot et al., 1997) and water/uncharged glycerol 1-monooleate bilayer (Pohorille et al., 1996). The intriguing possibility of anesthetics indirectly influencing membrane channel functions through inhomogeneous distribution of anesthetics in the lipid has recently been proposed by Cantor (1996, 1997). One should also consider the possibility that the inhomogeneous distribution of anesthetics across the membrane may result in specific site(s) of interaction at the lipid/protein interface. The lipid interface with proteins may play an important role in enabling the function of membrane protein (Dreger et al., 1997; Sunshine and McNamee, 1994). Thus the presence of anesthetics distributed in the lipid membrane may bring about changes in lipid-protein interactions (Firestone et al., 1994).

Molecular dynamics (MD) studies of small solutes in model bilayers touched upon the interplay between the size, hydrophobicity, and shape considerations in the permeation process across the lipid bilayer (Marrink and Berendsen, 1994, 1996). Emphasis on the contribution of "hopping" to the diffusion of a small molecule in lipid was demonstrated in the MD study of benzene in a model lipid (Bassolino-Klimas et al., 1993, 1995). Huang et al. (1995) introduced a single trichloroethylene molecule into dioleoylphosphatidylcholine (DOPC) lipid bilayer represented by 12 lipid molecules per layer. The authors claimed to observe changes in the phase, *gauche* defect population, area per headgroup, and lateral mean square displacement. However, the lengths of the MD trajectories are too short (200 ps each) and the size of the system too small for the results to be considered as definitive. Indeed, numerous studies have demonstrated that much longer runs are needed to reach an equilibrium structure for lipid bilayers (Tu et al., 1995; Pastor, 1994; Marrink et al., 1996).

We have recently performed a MD simulation of the DPPC liquid crystal (L_α) phase (Tu et al., 1995) that extended over 1.5 ns. The bilayer was demonstrated to be stable, and the structural properties of the L_α phase compare favorably with the available experimental data (Nagle, 1993). For example, the average area/lipid and lamellar spacing are within the experimental uncertainty. Furthermore, the average position of specific carbons, the electronic density, and the order parameters are also in good agreement with experimental data.

We have recently formulated a potential function to describe halothane (Scharf and Laasonen, 1996). Employing

ab initio quantum mechanical calculations, we have established structural data for the halothane molecule, namely bond lengths and angles, energy profiles for torsional motion, and an estimate for the molecular dipole moment. The total intermolecular potential function, which includes electrostatic, repulsive, and dispersion interactions, was fitted to reproduce the density of liquid halothane and its heat of vaporization at physiological temperature. Finally, we have also undertaken MD simulation of halothane solvated in water to investigate its structure and behavior in bulk solution (Scharf et al., manuscript in preparation).

In this study, we use MD simulations to probe the changes induced in a model DPPC lipid bilayer due to the introduction of a relatively low concentration of halothane. The simulations were performed in the liquid crystal phase of DPPC, which is the only phase relevant to biological applications. This phase is exhibited for DPPC above $T = 42°C$. Our results are intended to probe the dynamics of halothane in a model of a physiological membrane, in the sense that we address the correct physiological phase of biological membranes, even though technically the simulation is performed at a higher temperature.

Clinically relevant concentrations of general anesthetics are expressed as the minimum alveolar concentration (MAC) required to render 50% of the subjects anesthetized. According to Franks and Lieb (1979) and Craig et al. (1987), estimates of 1 MAC of halothane in DPPC are around a mole ratio of 0.025 (n_{hal}/n_{DPPC}). Here the simulation cell contains 64 lipid molecules, so that a 0.025 mole ratio implies only one to two halothane molecules. However, to improve the statistics of our calculation and sample more efficiently the anesthetic behavior, we have introduced four molecules into the bilayer. The resulting calculation corresponds to a molar concentration $\simeq$0.06, or 2–3 MAC.

The results presented in this paper are based on a 1.6-ns MD trajectory after the initial equilibration run. They provide information on the size and type of effects on a model membrane caused by anesthetics. In the following section, we will describe the setup of the system and the simulation methodology employed. Then results on the location and the dynamical behavior of the anesthetic will be presented and discussed. The structural properties of the lipid bilayer with halothane are compared to those of the pure lipid (Tu et al., 1995) and various experimental data mentioned above.

METHOD

The intermolecular potential used for the DPPC molecules is similar to that used in the previous study of the fully hydrated gel and liquid phases of the lipid bilayer (Tu et al., 1995, 1996). The rigid three-site SPC/E potential (Berendsen et al., 1987) was used for water. The potential for halothane is the flexible all-atom model developed recently by Scharf and Laasonen (1996) (see Table 1). The potentials

TABLE 1 Intermolecular potential parameters for halothane: charges (q) and Lennard-Jones parameters (σ, ϵ)

Site	q (e)	σ (Å)	ϵ (kcal/mol)
C_1	0.66	3.40	0.0765
F	−0.22	2.80	0.0735
C_2	0.215	3.40	0.0765
Br	−0.16	3.70	0.2941
Cl	−0.18	3.50	0.2206
H	0.125	2.75	0.0199

The Lorentz-Bertholot combining rules are used to calculate the Lennard-Jones parameters for the cross-interactions: $\sigma_{ij} = (\sigma_{ii} + \sigma_{jj})/2$; $\epsilon_{ij} = (\epsilon_{ii}\ \epsilon_{jj})^{1/2}$ (see also Scharf and Laasonen, 1996).

are based on energy minimization within the Car-Parrinello scheme of density functional theory to obtain the gas phase structure and potential parameter fitting to reproduce bulk halothane properties at room and physiological temperatures. It has been used for this study without further modification. The intermolecular Lennard-Jones parameters for the lipid-anesthetic interactions were obtained by using the classical Lorentz-Bertholot mixing rules.

The initial system was set up starting from a well-equilibrated configuration of a DPPC bilayer (Tu et al., 1995), corresponding to the structure of a fully hydrated liquid crystalline phase. The bilayer contains 32 lipid molecules per leaflet and 1792 water molecules. This system, equilibrated at constant temperature ($T_{ext} = 50°C$) and pressure ($P_{ext} = 1$ atm), is characterized by a surface area per lipid of $A \simeq 61.8$ Å^2 and lamellar spacing $d \simeq 67.3$ Å.

Four halothane molecules were initially incorporated into the bilayer near the glycerol region, with a pair at each lipid/water interface. To avoid unfavorable repulsive contacts with the lipid molecules, the introduction of the anesthetic molecules was achieved through a series of short MD runs (20 ps each). First the halothane molecules were treated as point masses with zero charges and van der Waals parameters. For each consecutive short MD run, the molecules were "grown" in size by extending their intramolecular bonds. The intra- and intermolecular interactions were simultaneously rescaled from zero to one. This procedure allows one to incorporate the molecules without having to first create free volume, which likely would induce large perturbations of the bilayer structure.

The MD trajectory was carried out with three-dimensional periodic boundary conditions, initially for 100 ps at constant temperature ($T_{ext} = 50°C$) and volume. Then we generated a 1.6-ns trajectory at constant temperature ($T_{ext} = 50°C$) and pressure ($P_{ext} = 1$ atm) (NPT), using the hybrid MD algorithm developed by Martyna et al. (1994, 1996), which resulted in zero surface tension in the directions tangential and normal to the bilayer surface. We note here that biological membranes are characterized by an unstressed bilayer at a local free energy minimum. They can be regarded as curvature-dominated and therefore were studied here at the limit of vanishing surface tension. (Jähnig, 1996; Brochard et al., 1976). The extended system (ES) equations of motion were integrated by using an iterative

Verlet-like algorithm. The fictitious masses of the ES variables were chosen according to the prescription given by Martyna et al. (1992), with time scales of 0.5 ps for the thermostats and 1 ps for the volume and cell variables. The Nosé-Hoover thermostat chain length was five. The Ewald method was used to compute the electrostatic interactions, and the minimum image convention was employed to calculate the real-space part of the Ewald sum and the van der Waals interactions with simple truncation at 10 Å (Allen and Tildesley, 1989). Bonds involving hydrogen atoms were constrained using the SHAKE algorithm (Ryckaert et al., 1977).

The calculation required 2.5 cpu h/ps, using 16 processors in a parallel/vector mode on the Cray C90 computer at the Pittsburgh Supercomputing Center.

RESULTS AND DISCUSSION

Overall structure

The time evolution of the calculated area per lipid (A) and the lamellar spacing (d) of the bilayer are shown in Fig. 1. The values at time $t = 0$ are those of the initial configuration, after the constant volume run. They correspond roughly to those of the pure lipid in the liquid crystalline phase at $T_{ext} = 50°C$ (Tu et al., 1995). The values of the surface area per lipid, A in Fig. 1, have been calculated by dividing the instantaneous surface area (S) of the simulation system by the number of lipids per layer, namely 32.

Fig. 1 shows that the system first undergoes a small lateral expansion before relaxing to an equilibrium state. Simultaneously, the system contracts in the direction of the normal to the bilayer, i.e., the d spacing decreases. At equilibrium, the calculated surface area per lipid molecule and the lamellar spacing oscillate around $A = 63.6$ Å^2 per molecule and $d = 65.9$ Å, respectively, compared to $A = 61.8$ Å^2 and $d = 67.3$ Å for the pure DPPC bilayer (Tu et al., 1995). The bilayer exhibits fluctuations in both A (± 1.5 Å^2) and d (± 1.5 Å) of magnitudes similar to those found in the pure lipid L_α phase simulation (Tu et al., 1995). Even though there are large fluctuations, the simulation shows unequivocally that the surface area increases slightly, and the lamellar spacing decreases slightly. Similar findings were reported by Trudell (1977) based on his analysis of a membrane fluidity increase. In contrast, based on x-ray and neutron data, Franks and Lieb (1979) reported no significant difference in the membrane structure in the study of the effects of anesthetics in lecithin/cholesterol bilayers at clinical concentrations.

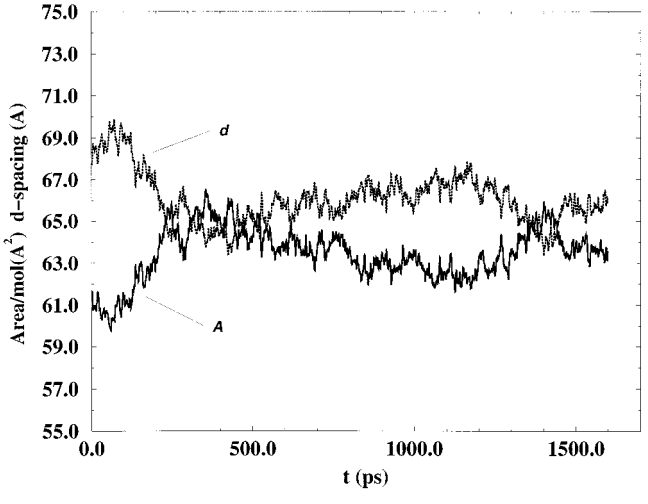

FIGURE 1 Time evolution of the area, A, per lipid in Å^2 (*solid line*) and lamellar spacing, d, in Å (*dotted line*) during the NPT MD simulation.

We also calculated the total volume, V, of the simulation system under consideration and compared it to the total volume in the pure lipid simulation of Tu et al. (1995). The corresponding average values are $V = (134.6 \pm 1.5)$ nm^3 and $V = (134.1 \pm 0.8)$ nm^3, respectively. Thus there is only a 0.4% increase due to the presence of a 6.5% mole fraction of halothane in the system. This volume change is in rough agreement with the measurements made by Franks and Lieb (1981). In fact, considering that the partial volume (in solution) of the halothane molecule is $\simeq 120$ cm^3/mol, the volume occupied by the four molecules should amount to 0.8 nm^3, which correlates well with the modest volume expansion from the simulation. This observation indicates, in agreement with Franks and Lieb (1981), that the bilayer expansion is almost wholly accounted for by the volume of the halothane molecules themselves and does not involve the creation of any additional free volume.

Location of halothane

Snapshots of the system for the configurations at time $t = 0$, 0.5 ns, 1.0 ns, and 1.5 ns of the NPT run are displayed in Fig. 2. Fig. 2 indicates that on the nanosecond time scale of the MD simulation, three of the halothane molecules remained in the vicinity of their initial positions. The fourth molecule migrated from the headgroup region to the middle of the bilayer. This fourth molecule also diffused parallel to the plane of the bilayer. The trajectories of the anesthetic molecules within the bilayer are depicted in Fig. 3, where both the perpendicular (Z) and the lateral (XY) traces of motions are displayed. As already noted, only one molecule (4) diffused to the center of the bilayer ($Z = 0$), whereas the others (see Fig. 3 A) remained near the glycerol backbone region ($Z = \pm 10$ to 15 Å). The lateral, in-plane trajectories shown in Fig. 3 B indicate that the motion of the halothane molecules is mostly confined to "rattling in a cage." This description is also valid for molecule 4, which "jumps" from its initial location to a site closer to the bilayer center and remains there for the duration of the simulation.

The present finding raises a fundamental question about the nanosecond time scale dynamics of the anesthetic molecules within the bilayer. In fact, the "rattling in a cage" motion is similar to the overall motions of the lipids them-

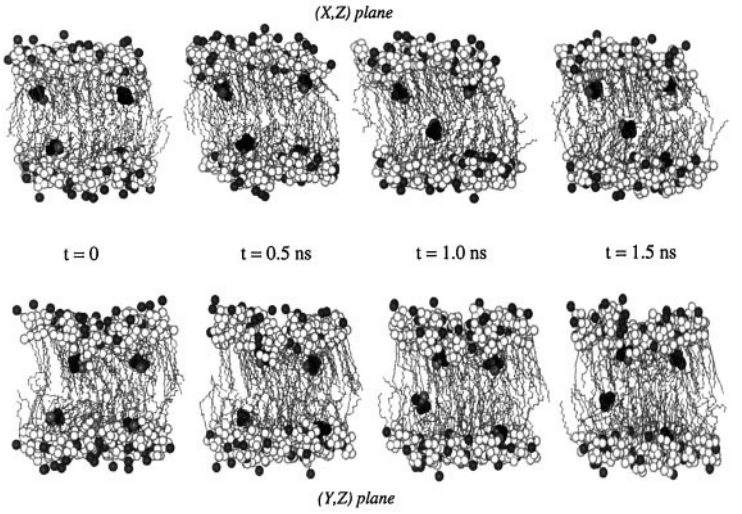

FIGURE 2 Orthogonal views of instantaneous configurations taken from the MD simulation, upper (*X,Z*) and lower (*Y,Z*), where *Z* is the bilayer normal. From left to right: (*A*) Initial configuration; (*B*) 0.5 ns; (*C*) 1.0 ns; (*D*) 1.5 ns. The halothane molecules are represented by their atomic van der Waal radii. The lipid molecules are shown in a ball-and-stick representation, except for the O (*white*) and N (*black*) atoms of the lipid headgroups. H atoms of the lipid and water molecules are omitted for clarity. Note that one of the halothane molecules diffuses to the bilayer center between *B* and *C*.

selves in the liquid crystalline phase (Tobias et al., 1997; Konig et al., 1992; Pastor and Feller 1996; Marrink and Berendsen, 1996).

A natural question to ask is whether the motion of an individual halothane molecule is simply a consequence of a plausible strong "binding" to a specific lipid molecule. To address this question, we display in Fig. 4 a superposition of snapshots of the four halothane molecules, along with their corresponding closest lipid molecules. Fig. 4 shows that the anesthetic molecules are indeed localized but are not "bound" with specific fixed orientations to a particular atom in the lipid molecule.

The motion of the halothane molecules has been further analyzed by monitoring the angle θ between the halothane molecular C-C axis and the bilayer normal. Fig. 5 shows that regardless of their location, all of the halothane molecules exhibit large-amplitude oscillations superimposed on end-on-end rotations. Our observations suggest that the motion of the halothane molecules in the DPPC bilayer is similar to that of small molecules (mol wt <50) in the lipid tail region (Marrink and Berendsen, 1996) and to benzene in a DMPC bilayer (Bassolino-Klimas et al., 1993, 1995). In their MD simulation, Bassolino-Klimas et al. considered four benzene molecules in a 36-molecule bilayer and analyzed the solute diffusion from a 4-ns simulation. As in the present study, the benzene molecules were found to rattle within voids in the bilayer and occasionally "jump" to a new location. These and our simulation results support the suggestions of Lieb and Stein (1969, 1971), who argued that the interior of a lipid membrane is inhomogeneous, and that the diffusion of a small solute within the membrane is analogous to diffusion through a soft polymer.

Analysis of the motion of the two molecules that are closest to the water/lipid interface (2 and 3) did not reveal any specific orientation with respect to the bilayer normal (Fig. 5). This finding is apparently in contradiction to the work of Yoshida et al. (1989). In their study, proton and ^{19}F-NMR shifts were used to estimate the affinity of the hydrophilic (—CHClBr) and hydrophobic (—CF_3) groups of the halothane molecule for the sodium dodecyl sulfate (SDS) micelle/water interface. From the magnitude of the affinity ratios they concluded that the halothane molecule adsorbs perpendicular to the micelle/water interface. SDS is an ionic system, and hence the micelle/water interface is likely different from the DPPC membrane lipid/water interface (Shelley et al., 1990). The present MD simulation, which suggests that the anesthetics under study present no specific orientation with respect to the interface, does not necessarily contradict the finding of Yoshida et al. (1989). This conclusion was for anesthetics interacting with micelles of a surfactant, which may very well exhibit different behaviors compared with membrane bilayer lipids. Although these studies were conducted at ambient temperature, we notice that our studies were conducted at 50°C, which may influence the motion of the anesthetics. However, we believe that the absence of a specific interaction for the anesthetics with the membrane will hold even at lower temperatures (see also in the discussion of halothane hydration).

Lipid structure

A convenient way to characterize the structure of the lipid bilayer is to calculate the electron density profiles from the MD trajectories. For a given configuration, the profile is calculated by placing a Gaussian distribution of electrons on each atomic center with a variance equal to the van der Waals radius. Averaging is then performed over the configurations from the MD trajectory. Fig. 6 displays the average profiles over the last 1.2 ns of the NPT run. The peak in the electron density profile corresponds to the polar headgroup region, and the trough at $Z = 0$ corresponds to the terminal methyl group region of the hydrocarbon chains. The overall structure is similar to that of a pure lipid bilayer in the liquid crystalline phase (Tu et al., 1995). One notes the broad

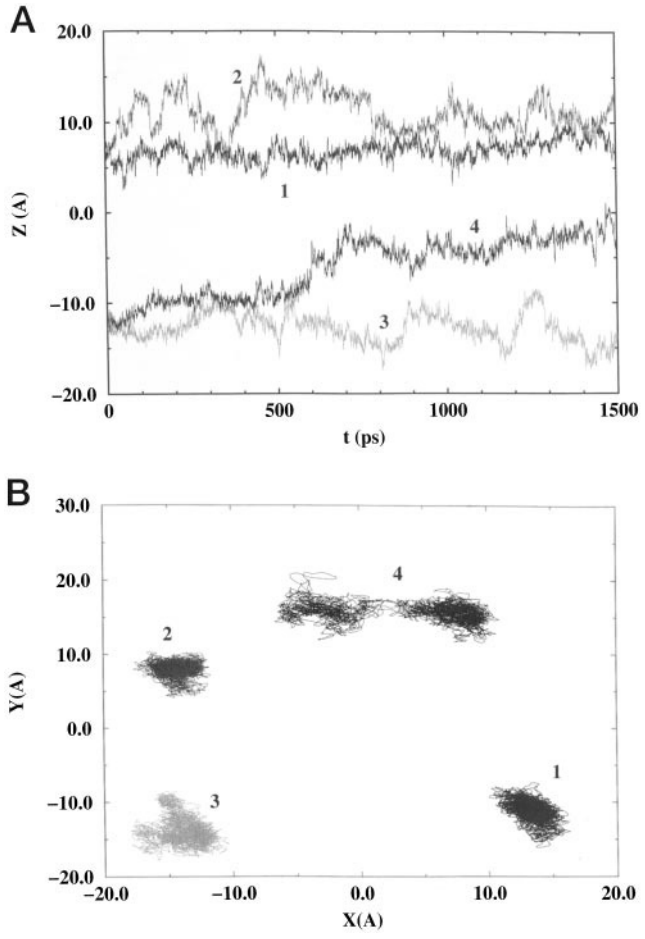

FIGURE 3 Center of mass coordinates as a function of time for the four halothane molecules (labeled 1, 2, 3, and 4) projected onto (*A*) the bilayer normal *Z* and (*B*) the *X,Y* plane.

distributions of the ammonium and the hydration of the acyl ester region. The distributions of the individual headgroup moieties appear to be different on each side of the bilayer. This is likely due to the fact that the number of halothane molecules in the headgroup region is not the same at the two interfaces. The higher concentration region ($Z > 0$) presents a symmetrical bimodal distribution of the ammonium group, somewhat characteristic of the gel phase of DPPC (Tu et al., 1996). It suggests that the presence of the anesthetic in excess in the headgroup region may have a direct effect on the orientation of the lipid dipole moment. However, one should keep in mind that the system under study is small and the length of the simulations is likely too short to allow the halothane molecules to completely sample the lipid bilayer. It is then more appropriate when comparing the density profiles to the experiment at the same concentration to consider a profile averaged over both monolayers.

We have calculated the distances from the bilayer center of different headgroup atoms (along the bilayer normal) averaged over both monolayers and compared them to the pure DPPC bilayer simulation results (Tu et al., 1995). For the headgroup ammonium methyl carbon atoms C_γ, the choline methylene carbons C_β and C_α, and the glycerol methylene carbon atoms GC-3, the average distances are (19.2 ± 4.0) Å, (19.2 ± 3.3) Å, (19.2 ± 2.6) Å, and (17.2 ± 2.1) Å, respectively, compared to (21.1 ± 3.0) Å, (20.3 ± 2.6) Å, (20.2 ± 2.4) Å, and (17.4 ± 2.1) Å, for the pure L_α phase. The observed differences, which are consistent with the small reduction in the lamellar spacing shown in Fig. 1, are confined to the headgroup region. We have also calculated the distances from the center of the bilayer for C4, C5, C9, C14, and C15 atoms of the acyl chains. The resulting values are (11.4 ± 1.7) Å, (10.9 ± 2.2) Å, (6.7 ± 2.3) Å, (2.5 ± 3.6) Å, and (1.8 ± 3.6) Å, respectively, compared to (11.7 ± 1.7) Å, (10.7 ± 1.8) Å, (6.9 ± 2.0) Å, (2.6 ± 2.0) Å, and (1.9 ± 2.1) Å for the pure lipid. In summary, although these are subtle differences, the overall density profiles in Fig. 6 are similar to previous results of pure lipid simulations. This finding likely explains why, in the experimental results of Franks and Lieb (1979), the presence of halothane at a low partial pressure did not appear to change the overall lipid bilayer structure.

The hydrocarbon chain behavior in the presence of halothane has been analyzed in terms of deuterium order parameters. In Fig. 7 we plot the order parameters calculated from the MD trajectory and compare them to the pure lipid data (Tu et al., 1995) for individual Sn-1 and Sn-2 chains of the lipid. For clarity, the error bars on the order parameters are not reported. The estimated standard deviations are of a magnitude similar to that of the pure lipid simulation, namely $\simeq \pm 0.02$. Fig. 7 shows that there is, within statistical error, no change in the order parameters along the chain due to the presence of the halothane molecules at the concentration under consideration. This observation is consistent with NMR results for halothane in the palmitoyloleoylphosphatidylcholine bilayer (Baber et al., 1995), which indicate at low anesthetic concentration no significant change in the segmental order of the lipid hydrocarbon chains. Specifically, the reported change of the order parameters due to the anesthetic was less than 10% up to a concentration of 40 mol%. We note that as for the pure lipid, the simulation predicts too much order at the two ends of the hydrocarbon chains compared to experiment (North and Cafiso, 1997).

We have also analyzed the chain conformations and found that there are 14, 22, 21, 19, 21, 20, 21, 21, 21, 23, 25, and 31% *gauche* defects in the last 12 bonds (C1–C2, . . . , C14–C15), respectively. The corresponding results for the pure lipid simulation (Tu et al., 1995) were 14, 25, 18, 23, 20, 20, 20, 23, 23, 25, 26, and 32%, respectively. Again the data show that at the present halothane concentration there is essentially no effect on the hydrocarbon chain conformation. This finding is in agreement with Raman studies of DMPC/cholesterol multilamellar vesicles containing halothane (Lieb et al., 1982), which suggested that the *trans-gauche* population ratios for the hydrocarbon chains were affected only at very high anesthetic concentration.

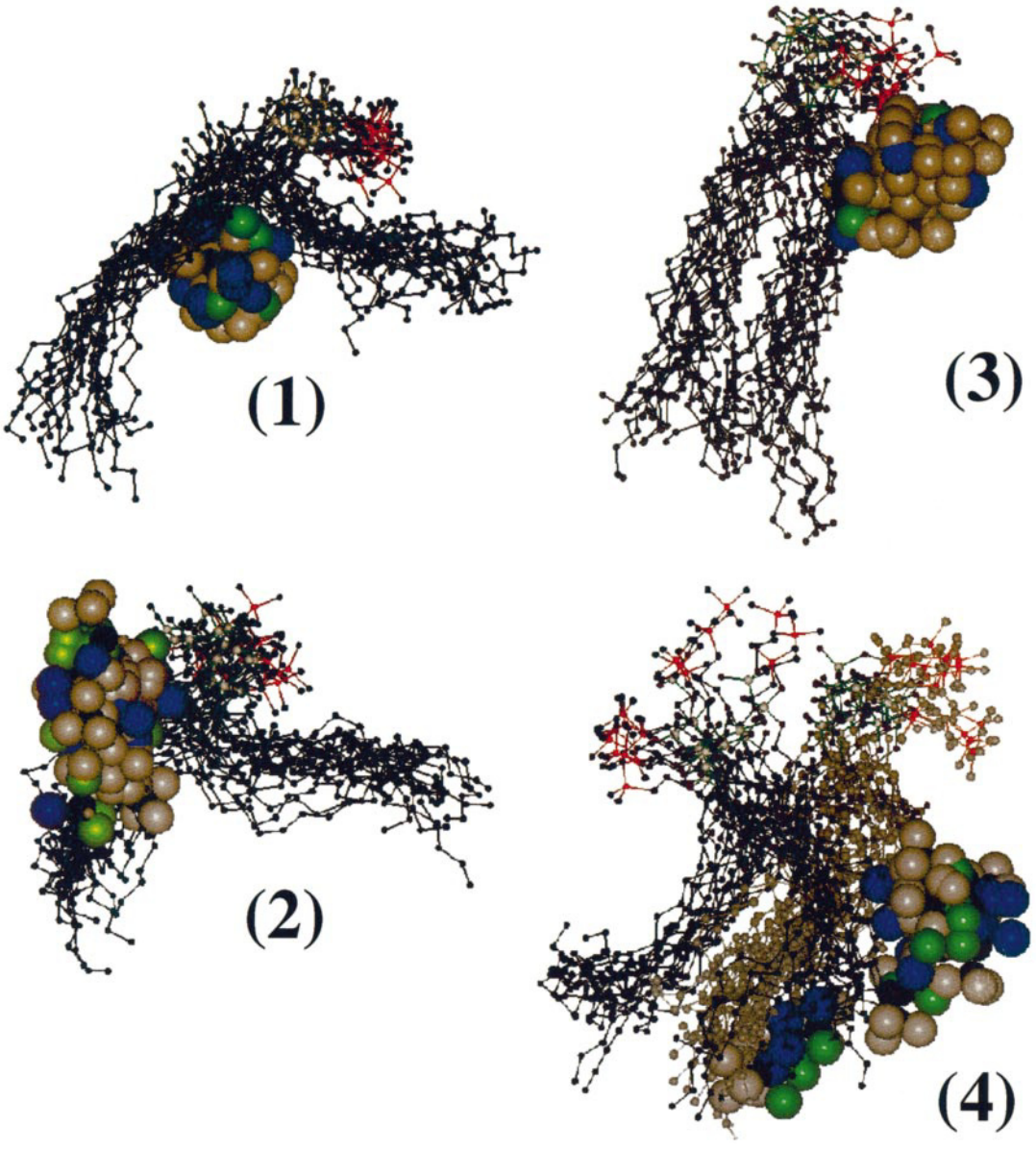

FIGURE 4 Superposition of configurations taken from the MD trajectory for the four halothane molecules and a close lipid neighbor, 1–3. Two neighboring lipids (*black* and *gray*) are shown for molecule 4. The halothane atoms are represented by their van der Walls radii with the following color scheme: F in gray, Cl in green, and Br in blue. DPPC is drawn in a ball-and-stick representation with atomic radii for N in red. H atoms are omitted for clarity.

Lipid and halothane hydration

As previously noted, the density profiles in Fig. 6 show that the water, as in the case of the pure DPPC L_α phase, penetrates into the glycerol ester region. To study the local hydration of the lipid molecules, we calculated the radial distribution functions $g(r)$ of water molecules around the phospholipid headgroup atoms. Fig. 8 shows $g(r)$ for the water molecule oxygen atoms around the headgroup ammonium nitrogen, the phosphate phosphorus, and the two glycerol ester carbonyl carbon atoms (C_{21} and C_{31}). We have also estimated the water coordination numbers associated with the respective atoms by integration up to the first minima in $g(r)$. These coordination numbers amount to 17.3, 5.6, 1.5, and 1.2 for N, P, C_{21}, and C_{31}, respectively. As expected from the $g(r)$ plots, there is essentially no difference in the hydration between the pure lipid and the lipid bilayer containing halothane molecules at the concentration under study.

The effects of anesthetics on the hydration of lipid headgroup moieties have been extensively studied experimentally. The DMPC/halothane system has been investigated at different water contents by gas chromatography and differential scanning microcalorimetry by Ueda et al. (1986). The authors suggested that anesthetics have a tendency to dehydrate the lipid bilayer. At high concentration, halothane decreased the ratio of bound water per lipid. Tsai et al.

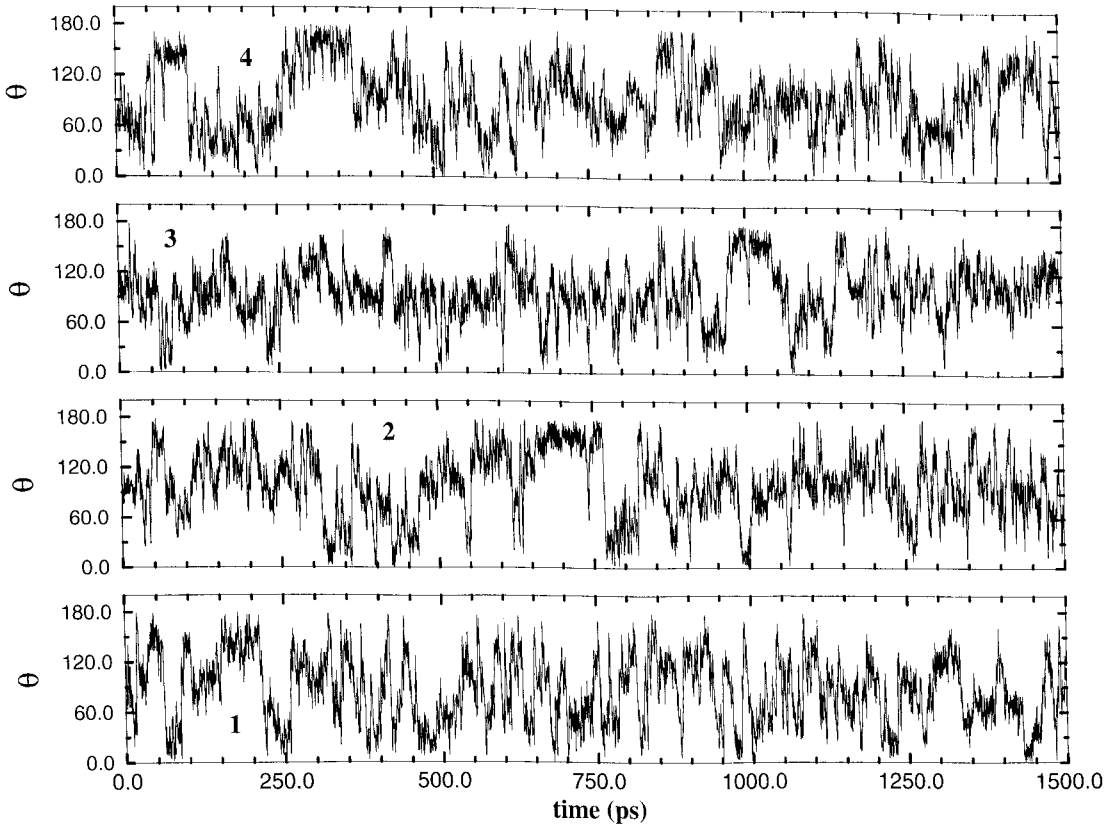

FIGURE 5 Time evolution of θ (degrees), the angle between the C–C axis of halothane molecules 1 to 4 (*from bottom to top*), and the bilayer normal.

(1987) used IR spectroscopy to probe the changes in the headgroup P–O and C═O stretching bands and the water O–H stretching bands for a DPPC/water reverse micelles in oil. The addition of halothane, to a 0.5 halothane/lipid mole ratio, induced changes in the P–O and O–H modes without affecting the C═O stretching band. The authors concluded

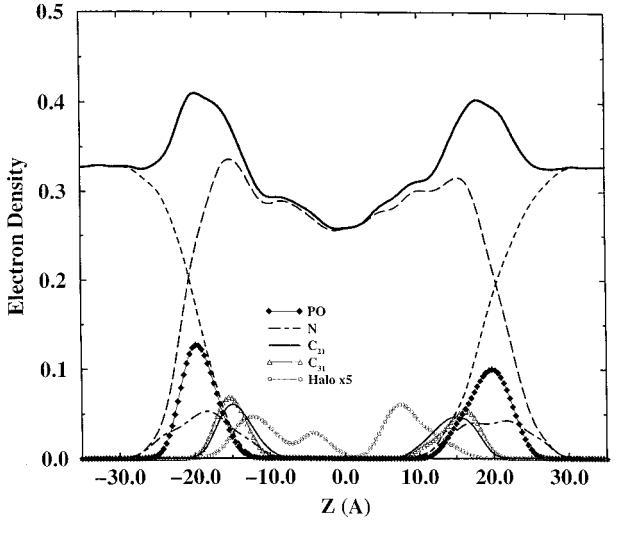

FIGURE 6 Electron density profiles along the bilayer normal, *Z*, averaged over the last 1.2 ns of the MD trajectory. The total density (*solid*), water (*dotted*), and hydrocarbon chain (*dashed*) contributions are indicated, along with those from the DPPC headgroup moieties. C_{21} and C_{31} are carbons of the acyl ester regions. The contribution from the halothane molecules has been enhanced by a factor of 5 for clarity.

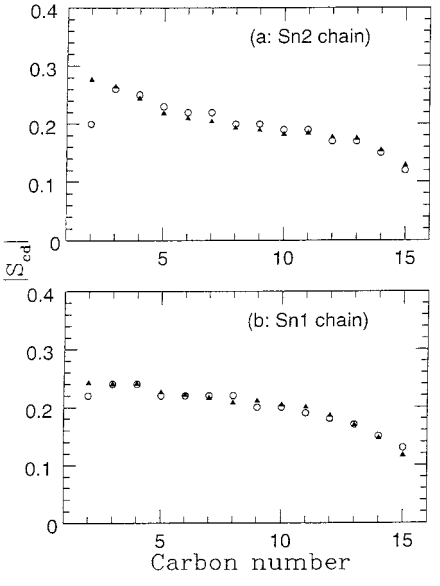

FIGURE 7 Deuterium order parameters for (*A*) the Sn1 and (*B*) Sn2 lipid hydrocarbon chains. The present results and pure DPPC (Tu et al., 1995) are shown as circles and triangles, respectively. The standard deviations ($\simeq \pm 0.02$ for both simulations) are omitted for clarity.

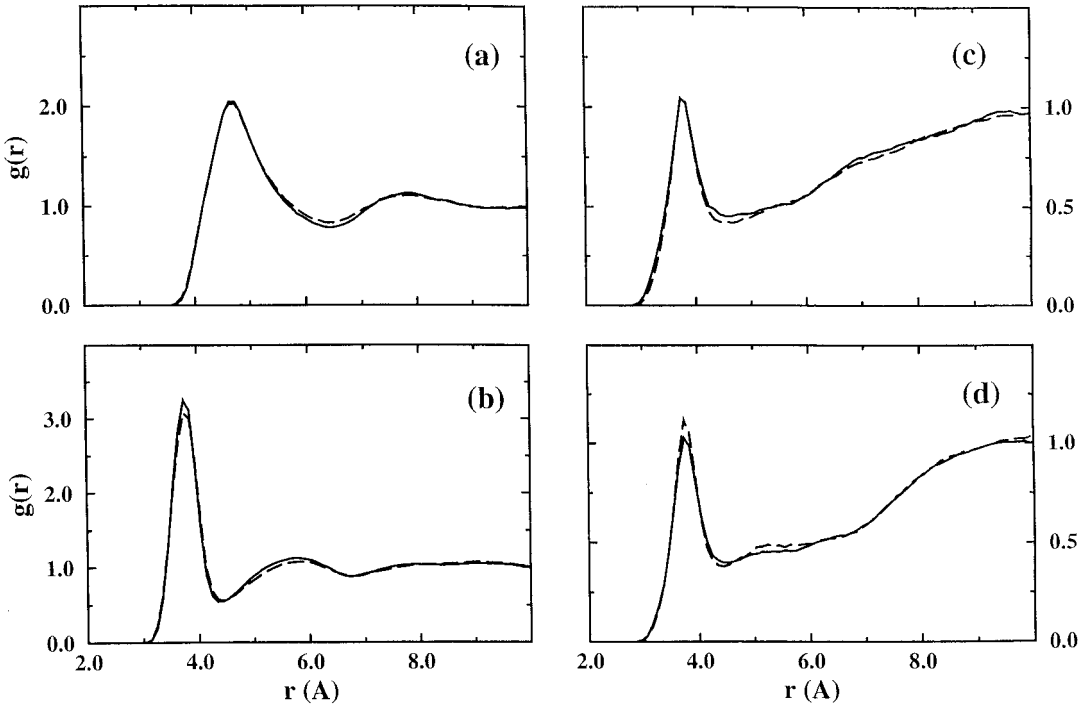

FIGURE 8 Radial distribution functions $g(r)$ for water molecule O atoms around the phospholipid headgroup and glycerol ester carbonyl carbon atoms (*solid lines*) compared to the data for the L_α phase of the pure lipid (Tu et al., 1995) (*dashed lines*). (*A*) N; (*B*) P; (*C*) C_{21}; (*D*) C_{31} atoms.

that the anesthetic action site is the phosphate group and that bound water molecules are released by the anesthetic. In a similar study, Tsai et al. (1990) followed the changes in P–O, $(CH_3)_3-N^+$, and C═O stretching modes of DPPC lipid vesicles in the presence of a 0.5 mole fraction of halothane. They also concluded that the anesthetic induced a release of the water, which was hydrogen-bonded to the phospholipid moiety.

To investigate the hydration of the anesthetic molecules in the lipid and quantify their accessibility to the water interface, we calculated $g(r)$ for water molecule oxygen atoms and the F, Cl, Br, and H atoms of halothane. Fig. 9

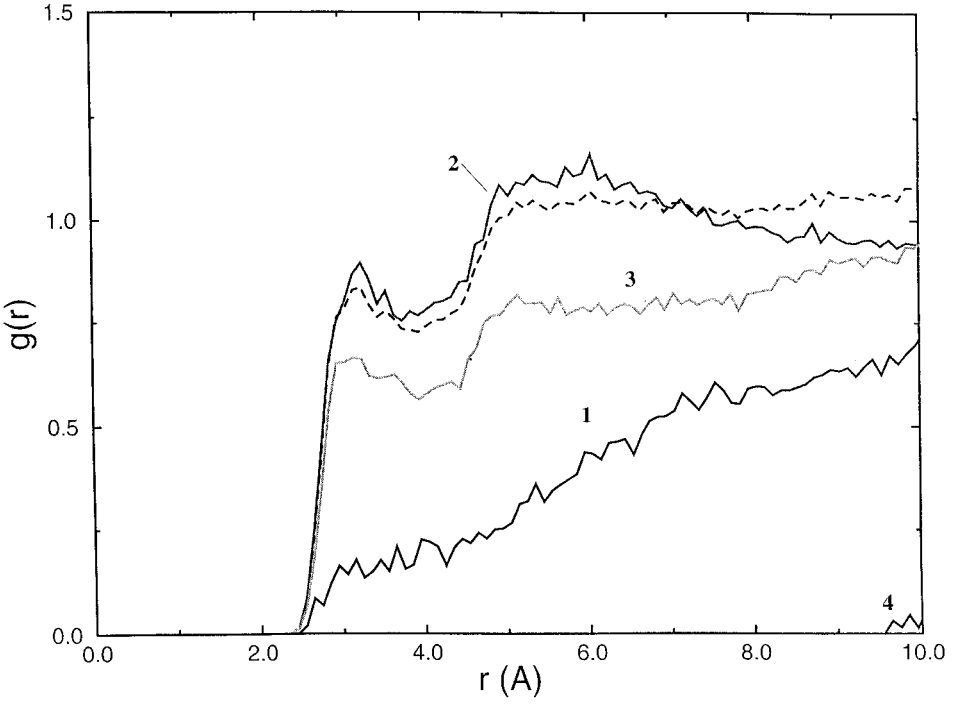

FIGURE 9 Radial distribution functions $g(r)$ of water molecule O atoms around the halothane F atoms. Shown are the results for molecules 1–4 of Fig. 3 *A*, along with data for halothane in bulk water (*dashed line*) (Scharf et al., manuscript in preparation). The contribution from halothane molecule 4 has been enhanced by a factor of 5 for clarity.

shows the O–F $g(r)$ for halothane molecules 1, 2, and 3 of Fig. 3 *A* and compares them to the $g(r)$ for halothane in bulk water (Scharf et al., manuscript in preparation). As expected, the O–F distribution is dependent on the halothane location. For molecule 4 of Fig. 3 *A*, which is located in the middle of the bilayer and has no contact with water molecules, $g(r)$ is null up to distances of 10 Å. Surprisingly, molecules 2 and 3, which are located near the interface but in the lipid (Fig. 3 *A*), have a hydration characteristic similar to that of halothane in bulk aqueous solution. Molecule 1, which is somewhat screened from the interface (Fig. 4), has less access to water molecules, and its corresponding $g(r)$ for O–F lacks a structured hydration shell. This analysis shows clearly that molecules 2 and 3 experience an environment that closely resembles that found in bulk aqueous solution. This observation is very consistent with the recent ^{19}F-NMR chemical shift analysis of anesthetic compounds in membranes (North and Cafiso, 1997; Tang et al., 1997). We note that it is the penetration of the water into the glycerol region in the liquid crystal phase, combined with the apparent localization of the halothane near that region, which gives the anesthetics an aqueous surrounding.

CONCLUSIONS

We have carried out a 1.6-ns MD simulation of DPPC in the liquid crystalline L_α phase containing a 6.5% mole fraction of halothane. The results of our simulation have been extensively compared to a variety of experimental data and to a previous simulation of the pure bilayer system (Tu et al., 1995). At the studied concentration, the overall bilayer structure undergoes subtle changes in the presence of the anesthetic. Specifically, we detect a small lateral expansion and a small contraction in the bilayer thickness. The overall volume expansion of the lipid/water system is found to be roughly comparable to the molecular volume of the halothane molecules introduced. In agreement with NMR experiments, there is no measurable change in the lipid hydrocarbon chain conformations. In contrast to what has sometimes been postulated in the literature, we found that halothane exhibits no specific binding to the lipid headgroup moieties, and no specific orientation with respect to the lipid/water interface. On the contrary, the anesthetic molecules partition into localized regions within the bilayer, and their motion within these volumes appears to be similar to that of other small solutes (Bassolino-Klimas et al., 1993, 1995). The apparent tendency of halothane to reside primarily in the headgroup region and to a lesser extent in the acyl tail region, but not in the acyl chain region, is striking, in light of theoretical hypotheses and complementary simulation studies (Cantor, 1997; Pohorille et al., 1996).

It is important to keep in mind the limitations of the present study. First, the length of the simulations precluded a complete sampling of the motion of the halothane molecules, which are likely to "visit" all regions of the bilayer on sufficiently long time scales. Our results are therefore not suitable for drawing reliable conclusions about partitioning of the anesthetic in the bilayer. Instead, they indicate at most only the nanosecond time scale description of events taking place in the bilayer. The configurations of the halothane/lipid "complexes" represented in Fig. 4 show the versatility as well as the potential complexity of such systems.

Another possible difficulty when comparing with experiments may arise because the present bilayer structure is actually a multilayer stack. The multilamellar structure is characterized by an aqueous environment different from a single "isolated" membrane, such as those used in some experiments.

In summary, the present MD simulation has elucidated the size and nature of the effects of halothane on a DPPC bilayer at concentrations close to clinical levels. The lack of specific binding to the lipid molecules, and the subtle effects on the lipid overall structure, as well as the spatial distribution found at this concentration, support the bulk of modern experimental observations on general anesthetic in membranes. The current focus of general anesthesia is on modifications of the structure and function of proteins. The present study is a necessary prelude to a more ambitious project involving the interaction of GAs with membrane proteins.

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